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THE INFLUENCE OF WHITE LIGHT ON 7 ISOLATES OF *ASPERGILLUS FLAVUS* LINK

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INTRODUCTION

While studying the morphogenetic action of light on several species of *Aspergillus* we have found an intraspecific variation which deserves attention. The present paper reports the responses of 7 cultures of *A. flavus* to several intensities of white light, and to different cycles of alternating light and darkness, as well as the minimal intensity and length of a photoperiod producing a phenomenon.

MATERIAL AND METHODS

Organisms: *A. flavus* 28-A, one of the strains isolated from local soils; STG-2, idem; 271, from pea seeds; Az-1, Az-2, Az-7, Az-9, from the collection of the Agricultural Faculty in Zemun.

Media: Czapek's solution agar with 3% sucrose, and Raulin's neutral solution agar. Amount of medium: 30 ml/Petri dish.

Inoculum: single spore cultures incubated 48 h on the corresponding medium (Czapek's or Raulin's)and transferred to the definitive dishes.

Irradiation system: The irradiation system consisted of incandescent lamps mounted in a water-cooled glass tank, as described and figured in a previous work (Muntanjola—Cvetković 1967). The lamps were of 60, 100 and 200 W, the intensity, as well as the number of them, depending on the intensity of the illumination period required

Intensities and periods of illumination: White light 2.000 lux (185 f.cd.) 12 h + darkness 12 h daily; 2.000 lux (185 f.cd.) 15 minutes daily; 50 lux (4,6 f.cd.) 4 h + darkness 20 h daily; 50 lux (4,6 f.cd.) 1 h + darkness 23 h daily.

Incubation time: 8 days after transfer of the 48 h old incipient colonies to the definitive petri dishes.

Incubation temperature: 25 ± 1°C.

RESULTS

A. MORPHOGENETIC RESPONSES TO LIGHT

All the strains of *A. flavus* here studied have shown some kind of sensitiveness to the light, though the responses to the energy received were different in quality and quantity.

Two of the strains, 28-A and STG-2, have shown the greatest sensitiveness, their capacity to form the conidial apparatus being strongly dependent on the intensity and quality of irradiation, as well as on the length of the illumination periods. A colony incubated in the dark presents a very characteristic aspect, where growth is strictly or almost strictly mycelial; the mycelium is more or less abundant depending on the medium, though sporulation is totally absent or very poor; instead, the vegetative hyphae form the dense masses of pseudoparenchymatous tissue called sclerotia; these sclerotia, at first white and later on brown, occupy the whole surface of the colony. The magnitude of sclerotial increase has been found negatively correlated with the light received: the best development of conidia and sclerotia are obtained under the opposite conditions. As the colony receives a certain amount of light the vegetative growth is checked and the mycelium iniciates a differentiation which ultimately produces a zone of conidial apparatus even if the colony is again placed in the dark. If the colony does not receive any other light-stimulus no more rings are to be formed; mycelial growth or sclerotial production will succeed without more zonations. So, under alternating periods of light and darkness colonies exhibit a more or less definite zonation, depending on the intensity of the energy received during the illumination period.

A. flavus Az-1 is likewise a sclerotium-forming strain. Though these structures are not abundant here and develop later than in 28-A and STG-2, they have only been observed in colonies grown in darkness or under conditions of deficient light. In this isolate, as well as in Az-2, Az-7, Az-9, and 271, the morphogenetic responses to the light are much less spectacular. The conidial production in darkness has been comparable to that from colonies kept in light of different intensities, but the quantity of mycelium, pigment in the reverse of the colonies, length and density of the conidiophores, etc., have not been the same. That means that in these cases, though sporulation is not suppressed by darkness, the colonies under this condition are nevertheless distinguishable from those maintained in light, principally by the amount of mycelium, which in the dark overgrows the surface and forms white mycelial patches. In these strains, too, more or less conspicuously zonate colonies may result from alternating periods of light and darkness; zonation is here constituted by rings richer in mycelium which supports as lateral branches the fertile structures, and rings where the vegetative mycelium is scanty and the coni-

diophores arise from the agar surface. The conidial heads formed in light are well developped and radiate. In the strain 271, besides this richer amount of mycelium in the dark, a stronger production of pigment in the reverse of the colonies is to be observed when light is lacking.

In any case we have found that the diameter of the colonies is much more influenced by temperature than by light or darkness. As we have pointed out in an other paper (Muntanjola—Cvetković 1967) each fungus has a typical curve of growth rate for each medium: light increases only slightly the diameter of the colonies, though cultures kept in darkness show a distinct tendency toward a richer amount of aerial mycelium.

B. INTENSITY AND LENGTH OF LIGHT EXPOSURE

Conidiophore differentiation occurs as long as any white light is present. Under the conditions of our experiments, sporulation of the strains 28-A and STG-2 — which does not occur in the dark — has been obtained on Czapek's solution agar under the regimes of white light 50 lux (about 5 f.cd.) 1 h daily, and white light 2.000 lux (185 f.cd.) 15 min. daily. In both cases the production of the conidial apparatus is rather poor, but still it is always richer than in darkness; the concentric zones are not very conspicuous and they are limited to the marginal areas.

As the intensity and length of light exposure increase, the conidial production is stronger and zonation more evident. Under the regime of white light 2.000 lux 1 h daily the zonation is already remarkable and it is constituted by rings (4—5 in 8 days old cultures) of conidiophores and conidial heads, separated by 2 mm wide zones of sclerotia.

Upon Raulin's solution agar zonation is by far less conspicuous than upon Czapek's agar, but here too we can observe how sporulation increases with the intensity of the illumination period. In both strains 28-A and STG-2 colonies developed in white light of 2.000 lux 15 min daily, and in white light 50 lux 1 h daily, showed a poor sporulation; still they were different from those grown in darkness, where the great abundance of sclerotia and the complete lack of conidial heads give the character to the colony. Upon Raulin's solution agar the results have been comparable between the colonies subjected to the following regimes: white light 2.000 lux 15 min daily, and white light 50 lux 1 h daily; but the difference has been evident when comparing these results and those obtained under whitle light 50 lux 4 h daily, where the sporulation has been much more important.

As for the other strains, the situation is in general the same; the differences are here observed in the quantity of sterile mycelium or floccosity of the colonies.

CONCLUSIONS

1. Among 7 isolates of *A. flavus* there has been found, in their morphogenetic responses to light, an intraspecific variation which deserves cognition. Some of these strains have shown a strong sensitiveness to light, this factor inducing the asexual sporulation; when growing in the dark these strains produce only mycelium and sclerotia, while conidial apparatus is completely lacking.

The effect of light energy on other strains has been weaker, though always measurable; here sporulation is not suppressed in the absence of light, but the amount of sterile mycelium is greater in the dark. Other effects, like a more intense pigmentation in the reverse of the colonies, smaller columnar heads, etc., may result from this condition.

That means that the strains here studied corroborate what was stated by Brown (1925) for some other fungi: »As sporulation is correlated with autolysis of mycelium and sclerotium formation connotes accumulation of mycelium, it is obvious that the two are in the main antithetic phenomena«. In this respect these strains would as well corroborate in some way the theory of Cochrane (1958), called by Carlile (1965) »the inhibition theory«, after which »there is in truth no such a thing as stimulation by light in the induction of *(asexual)* reproductive activity; instead, growth is checked and the chain of events so initiated leads to *(asexual)* reproduction if other factors are not limiting«.

2. A zonation of the colonies may result from alternating cycles of light and darkness, provided the illumination intensity is high enough. In general, the Czapek's solution agar favours zonation more than the Raulin's neutral solution agar. In the strong sclerotia-forming strains 28-A and STG-2 zonation is given by alternating rings of conidial apparatus and sclerotia; in the other strains zonation has in general been constituted by rings more floccose, where vegetative mycelium supports as lateral branches the fertile structures, and rings where the conidiophores arise from the substratum.

3. White light of an intensity of 50 lux (about 5 f. cd.) irradiating 1 h daily is enough to induce the conidiophore formation in the strains which do not sporulate in the dark or that do so very deficiently under this condition. At this level, neither sporulation nor zonation are intense on Czapek's solution agar +3% sucrose. Both phenomena begin to be conspicuous in colonies exposed to white light of 2.000 lux (185 f. cd.) 1 h daily, or to white light 50 lux 4 h daily.

No signifficant differences have been observed between colonies grown under the following regimes: white light 50 lux 1 h daily, and white light 2.000 lux 15 min daily; or between white light 2.000 lux 1 h daily, and white light 50 lux 4 h daily.

4. The phenomenon of conidial apparatus promotion decreased with the diminution of the intensity and length of the light exposure, in the following order:

White light 50 lux 4 h daily
White light 2.000 lux 1 h daily

White light 50 lux 1 h daily = White light 2.000 lux 15 min daily Constant darkness.

The amount of sterile mycelium in general, and that of sclerotia in the sclerotia-forming strains, increases in the opposite order.

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Rezime

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UTICAJ BELE SVETLOSTI NA 7 SOJEVA *ASPERGILLUS FLAVUS* LINK

U ovom radu su iznete reakcije 7 sojeva *A. flavus* na različite intenzitete bele svetlosti i na razne cikluse svetlost—mrak, kao i podaci o minimalnom intenzitetu i dužini fotoperioda koji mogu da izazovu izvestan fenomen.

Među ovim sojevima, izolovanim iz lokalnih zemljišta, postoji, u odnosu na njihovu morfogenetsku reakciju na svetlost, intraspecifična varijacija. Neki sojevi pokazuju jaku osetljivost prema svetlosti, koja stimulira aseksualnu sporulaciju; kada se gaje u mraku ovi sojevi daju samo micelijum i sklerocije, dok konidije potpuno izostaju. Efekat svetlosne energije na druge sojeve je slabiji, ma da je uvek merljiv; kod njih sporulacija nije sprečena u otsustvu svetlosti, ali je količina sterilnog micelijuma veća u mraku; drugi efekti, kao intenzivnija pigmentacija na donjoj strani kolonija, sitnije glavice sa konidijama koje su raspoređene u obliku stuba, itd., mogu da budu rezultat mraka.

To znači da sojevi koji su ovde ispitivani potvrduju ono što je konstatovao Brown (1925) za neke druge gljive: pošto je sporulacija u korelaciji sa autolizom micelijuma, a obrazovanje sklerocije predstavlja akumulaciju micelijuma, očevidno je da su ova dva fenomena uglavnom antitetička. U tom pogledu, sojevi bi bili na neki način povezani sa teorijom Cochrane-a (1958) koju je Carlile (1965) nazvao »teorijom inhibicije«, prema kojoj u suštini svetlost ne stimuliše pojave

(aseksualne) reproduktivne aktivnosti; umesto toga rastenje se obustavlja i niz događaja koji su na taj način započeti vode do *(aseksualne)* reprodukcije ako drugi faktori nisu ograničavajući.

Kao rezultat alternativnih ciklusa svetlosti i mraka mogu da se pojave zone na kolonijama, ukoliko je intenzitet osvetljenja dovoljno visok.

Bela svetlost intenziteta od 50 luksa za 1 sat dnevnog osvetljavanja je dovoljna da indukuje formiranje konidiofora kod sojeva koji nemaju sporulaciju u mraku, ili kod kojih je ona vrlo slaba u tim uslovima. Sa ovim intenzitetom sporulacija je slaba, a zone nisu jasno izražene. Obe pojave postaju upadljive kod kolonija izloženih beloj svetlosti od 2.000 luksa 1 sat dnevno, ili beloj svetlosti od 50 luksa 4 sata dnevno.

Obrazovanje konidijskog aparata opada sa smanjivanjem intenziteta i dužinom perioda osvetljenja. Količina sterilnog micelijuma uopšte i količina sklerocija kod sojeva koji ih formiraju, povećava se u suprotnom smislu .

(Iz Instituta za biološka istraživanja, Beograd)